3.1. Biological importance of selenadiazoles
Selenium containing heterocyclic system is of interest due to their potential pharmaceutical properties [1] and some of them are used as chemotherapeutic agents [2]. Among selenium-containing heterocyclic compounds, 1,2,3-selenadiazoles and their derivatives are well known and have attracted attention as versatile synthetic intermediates [3]. Many substituted 1,2,3-selenadiazoles have been prepared to-date and some of them show high antibacterial activity [4]. The antifungal activity of substituted 1,2,3-selenadiazoles has also been determined [5]. It has been found that the introduction of a 1,2,3-selenadiazole ring to molecules of known biological activity changes their activities and in some cases leads to an increase in their biological potential [6]. 1,2,3-selenadiazoles also exhibit varied other biological activities, including anticipated antitumor [7], antimicrobial [8], antiaflatoxigenic [9], antihaemostatic [10] and insecticidal activities [11].

3.2. Synthesis of 1,2,3-selenadiazole derivatives
Petrov et al. [12] reported the synthesis of 4-(2-hydroxy-5-methoxy- and -benzyloxy)-1,2,3-selenadiazoles 3 by treating 2-hydroxy-5-methoxyacetophenone semicarbazone 2 with selenium (IV) oxide in acetic acid under reflux condition. This semicarbazone 2 is obtained from the reaction of 2-hydroxy-5-methoxyacetophenone 1 with semicarbazide hydrochloride in the presence of sodium acetate in 2-propanol.

\[
\begin{align*}
\text{O} & \quad \text{NH₂NHCONH₂·HCl} & \quad \text{CH₃COONa} \\
\text{RO} & \quad \text{NH} & \quad \text{CONH₂} \\
\text{OH} & \quad \text{O} & \quad \text{S₉O₂/AcOH} \\
\text{1} & \quad \text{2} & \quad \text{3}
\end{align*}
\]

R = Me; PhCH₂

Karnik et al. [13] described the reaction between naphthopyranones 4 and semicarbazide hydrochloride in the presence of sodium acetate in dry ethanol to furnish the corresponding semicarbazones 5. The resulting semicarbazones 5 were further reacted with selenium dioxide in the presence of acetic acid under reflux condition to afford the 4H-naphtho[1',2':5,6]pyrano[3,4-d](1,2,3)selenadiazole derivatives 6 in good yields.
Maryanoff et al. [14] reported the synthesis of two regioisomers of 1,2,3-selenadiazole derivatives 9 and 10 from the reaction of unsymmetrical semicarbazones 8 with selenium dioxide.

Padmavathi et al. [15] reported the synthesis of 4-alkyl-5-phenyl-7-aryl-6,6-dimethoxycarbonyl/6-cyano-6-ethoxycarbonyl/6,6-dicyano-4,5,7-trihydrobenzo[d][1,2,3]-selenadiazoles 13 by the oxidative cyclization of their corresponding semicarbazones 12 with selenium dioxide in acetic acid at 70 °C [16]. This semicarbazones 12 were obtained from the reaction of 3-alkyl-2-phenyl-6-aryl-1,1-disubstituted cyclohexan-4-ones 11 with semicarbazide hydrochloride.

Saravanan et al. [17] described the synthesis of 5-(2-nitro-1-arylpropyl)-4-aryl-1,2,3-selenadiazoles 17 by the treatment of semicarbazones of Michael adduct 16 with selenium dioxide in THF under heating condition. This semicarbazones were prepared from the reaction of Michael adducts 15 with semicarbazide hydrochloride in the presence of sodium acetate and catalytic amount of phase transfer catalyst in
dioxane/water mixture. The Michael adducts 15 can be synthesised from reaction of nitroethane with chalcones 14 in the presence of sodium methoxide in methanol.

Bhaskar Reddy et al. [18] reported the synthesis of 4-aryl-1,2,3-selenadiazolyl-5-sulfanylacetic acid 20 via the oxidative cyclization of semicarbazones of phenacyl sulfanylacetic acid 19 with selenium dioxide in acetic acid medium. This semicarbazones 19 are prepared from the reaction of phenacyl sulfanylacetic acids 18 with semicarbazide hydrochloride in presence of sodium acetate and methanol.

Gopalakrishnan et al. [19] reported the one pot synthesis of 5,7-diaryl-4,4-dimethyl-4,5,6,7-tetrahydropyridino[3,4-\textit{d}]1,2,3-selenadiazoles 23 from the reaction of 3-dimethyl-2,6-diaryl-piperidin-4-ones, semicarbazide hydrochloride and selenium dioxide in the presence of NaHSO₄·SiO₂ as a heterogeneous catalyst in dry media under microwave irradiation.

Mousa et al. [21] reported the synthesis of multi-arm 1,2,3-selenadiazole derivatives 29 in high yield by the reaction between aromatic polyketones 27, p-toluenesulfonyl hydrazide and selenium dioxide in dioxane under reflux condition.

Hayat et al. [22] reported the synthesis of 1,2,3-selenadiazole 34 from the oxidative cyclization of novel 2-(quinolin-8-yloxy)acetohydrazones 33 using selenium dioxide in acetic acid. The key intermediates, 2-(quinolin-8-yloxy)acetohydrazone derivatives 33 were synthesized by the condensation of 8-quinolinoxyacetic acid hydrazide 32 with different aromatic ketones.
Atta et al. [23] described the synthesis of 1,2,3-selenadiazoles 37 from the reaction of semicarbazones 36 with selenium dioxide in acetic acid medium via the oxidative cyclization process.

\[ R = H, \text{OMe}; \; R' = \text{Me, Et}; \; R'' = \text{Me, Et} \]
3.3. Synthesis of mono and bis-1,2,3-selenadiazole derivatives 39 and 40 - The present work

The synthesis of mono and bis-1,2,3-selenadiazole derivatives 39 and 40 have been achieved from the action of selenium dioxide on the mixture of semicarbazones, which were obtained from the reaction of 2-(3-oxo-1,3-diarylpropyl)-1-cyclohexanone with semicarbazide hydrochloride.

Scheme 3.1. Synthesis of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

DiastereomERICALLY pure racemic cyclic 1,5-diketones, 2-(3-oxo-1,3-diarylpropyl)-1-cyclohexanones 38 were obtained via a simple Michael addition of cyclohexanone with substituted chalcones by a known method [24]. The synthesis of mono and bis-1,2,3-selenadiazole derivatives have been achieved from the action of selenium dioxide on the mono/bis-semicarbazones of ketones 38.

In the initial studies, the reaction of 2-(3-oxo-1-(2-naphthyl)-3-phenylpropyl)-1-cyclohexanone 38d with semicarbazide hydrochloride and sodium acetate in 1:1:1 ratio was carried out in ethanol under reflux for 3 h at 80 ºC to get the mono semicarbazone, in 78% yield. The presence of only one carbonyl signal in the $^{13}$C NMR spectrum of the product confirms the mono semicarbazone formation and the HMBC spectrum of the mono semicarbazone of 38d has contour connecting the aromatic hydrogens and the carbonyl carbon indicating that the derivatisation has taken place on cyclohexane ring. The oxidative cyclization of the mono semicarbazone with 2 equivalent of selenium dioxide in tetrahydrofuran was subsequently carried out. After completion of the reaction, the reaction mixture was filtered out to remove the selenium and then subjected to column chromatography to afford the mono-selenadiazole 39d in 70% yield. The reaction of 38d with semicarbazide hydrochloride and sodium acetate in 1:2.5:2.5 ratio afforded a mixture of mono and bis-semicarbazones, the latter one dominating. The bis-semicarbazone
has poor solubility in many of the popular NMR solvents preventing its unambiguous characterization. The oxidative cyclization has been carried out on the mixture of the semicarbazones without effecting the separation with three equivalents of selenium dioxide in tetrahydrofuran. The fact that two products, the mono selenadiazole 39d and the bis-selenadiazole 40d, were formed proved the formation of the bis-semicarbazone indirectly. Compounds 39d and 40d have been successfully separated out and as expected, the yield of 40d is more than that of 39d in this reaction. The reaction was then carried out with some other substrates by the above optimized sequential two step process and the details are presented in Table 3.1 and Scheme 3.1. The semicarbazone prepared by the reaction of 38d with semicarbazide hydrochloride and sodium acetate in 1:4:4 ratios on further treatment with three equivalents of selenium dioxide in tetrahydrofuran afforded exclusively bis-selenadiazole in 79% isolated yield.

### Table 3.1. Yields of 39 and 40 by employing different mole ratio of semicarbazidehydrochloride

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ar</th>
<th>Ar'</th>
<th>Yield (%)a</th>
<th>1 equiv. of semicarbazide</th>
<th>2.5 equiv. of semicarbazide</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>C₆H₅</td>
<td>C₆H₅</td>
<td>75</td>
<td>--</td>
<td>12</td>
</tr>
<tr>
<td>b</td>
<td>C₆H₅</td>
<td>4-ClC₆H₄</td>
<td>76</td>
<td>--</td>
<td>13</td>
</tr>
<tr>
<td>c</td>
<td>C₆H₅</td>
<td>4-MeOC₆H₄</td>
<td>66</td>
<td>--</td>
<td>17</td>
</tr>
<tr>
<td>d</td>
<td>C₆H₅</td>
<td>2-Naphthyl</td>
<td>70</td>
<td>--</td>
<td>20</td>
</tr>
<tr>
<td>e</td>
<td>4-MeC₆H₄</td>
<td>C₆H₅</td>
<td>69</td>
<td>--</td>
<td>15</td>
</tr>
<tr>
<td>f</td>
<td>4-ClC₆H₄</td>
<td>C₆H₅</td>
<td>72</td>
<td>--</td>
<td>10</td>
</tr>
<tr>
<td>g</td>
<td>4-ClC₆H₄</td>
<td>4-MeC₆H₄</td>
<td>70</td>
<td>--</td>
<td>12</td>
</tr>
<tr>
<td>h</td>
<td>4-ClC₆H₄</td>
<td>4-ClC₆H₄</td>
<td>75</td>
<td>--</td>
<td>20</td>
</tr>
<tr>
<td>i</td>
<td>C₆H₅</td>
<td>4-MeC₆H₄</td>
<td>79</td>
<td>--</td>
<td>12</td>
</tr>
<tr>
<td>j</td>
<td>4-MeOC₆H₄</td>
<td>C₆H₅</td>
<td>76</td>
<td>--</td>
<td>15</td>
</tr>
<tr>
<td>k</td>
<td>4-ClC₆H₄</td>
<td>4-MeOC₆H₄</td>
<td>78</td>
<td>--</td>
<td>15</td>
</tr>
</tbody>
</table>

*Isolated yield after purification by column chromatography.

bNo product was formed.

The structures of the mono-1,2,3-selenadiazoles 39 were established from ¹H, ¹³C and two dimensional NMR spectroscopic data as illustrated for a representative example, 39g (Figure 3.1). In the ¹H NMR spectrum of mono-1,2,3-selenadiazole 39g, the H-2' protons shows two doublet of doublets at 3.56 ppm (J = 17.1 and 8.4 Hz) and 3.83 ppm (J = 17.1 and 5.4 Hz), which shows H,H-COSY correlation with the triplet of...
doublet at 3.96 ppm ($J = 8.4, 5.4$ Hz), assignable to H-3', and HMBC with C-4 at 39.9, C-3' at 44.0, C-1'' at 141.5 and carbonyl C-1' at 198.0 ppm. The H-3' proton further shows C,H-COSY correlation with C-3' at 44.0 ppm, H,H-COSY correlation with the multiplet of H-4 proton at 3.70-3.76 ppm and HMBCs with 24.6, 39.9, 42.9, 141.5 and 129.7, the latter all assignable to C-5, C-4, C-2', C-1'' and C-2''' respectively. The H-4 proton exhibits: (i) H,H-COSY correlation with the multiplet at 1.59-1.80 ppm, which is due to H-5 protons and one of H-6 proton, (ii) C,H-COSY correlation with C-4 at 39.9 ppm and (iii) HMB correlations with C-2' at 42.9 ppm and C-3a at 157.8 ppm. The two cyclohexyl H-5 and one H-6 protons appear as a multiplet at 1.59-1.80 ppm, which exhibits C,H-COSY correlation with C-5 at 24.6 ppm due to H-5 protons and HMBCs with 19.7, 25.2, 39.9 and 44.0 ppm ascribable to C-6, C-7, C-4 and C-3'. The multiplet at 1.94-2.04 ppm accounting for the left out one of the two H-6 protons show: (i) H,H-COSY correlation with two doublet of triplets of H-7 protons at 2.91 ($J = 18.0$ and 6.6 Hz, axial like) and 3.05 ($J = 18.0$ and 5.1 Hz, equatorial like), (ii) C,H-COSY correlation with C-6 at 19.7 ppm. The H-7 proton also shows HMBCs with C-6 at 19.7, C-5 at 24.6 and C-3a at 157.8 ppm (Figure 3.2 to 3.12).

**Figure 3.1.** Selected HMBCs and $^1$H and $^{13}$C chemical shifts in compound 39g
Figure 3.2. $^1$H NMR Spectrum of 39g (CDCl$_3$)

Figure 3.3. $^1$H NMR Spectrum of 39g (expanded)
Figure 3.4. $^{13}$C NMR Spectrum of 39g (CDCl$_3$)

Figure 3.5. DEPT Spectrum of 39g (CDCl$_3$)
Chapter 3

Results and discussion

Figure 3.6. H,H-COSY Spectrum of 39g

Figure 3.7. H,H-COSY Spectrum of 39g (expanded)
Figure 3.8. HMBC Spectrum of 39g

Figure 3.9. HMBC Spectrum of 39g (expanded)
Figure 3.10. HMBC Spectrum of 39g (expanded)

Figure 3.11. C,H-COSY Spectrum of 39g
The structure of mono-selenadiazoles determined from X-ray crystallographic study for the single crystal of 39b and that assigned by NMR spectroscopic studies are in good agreement. The ORTEP diagram [25] of 39b (Figure 3.13) shows that both H-1' and H-4 hydrogens are in a trans relationship with respect to C-1'-C-4 bond and the coupling constant between these two hydrogens is 8.4 Hz. The crystal structures of 39d and 39e have also been solved [26]. The mass spectrum for the compound 39e is obtained as m/e 409.0824 [M-1] (calcd. 410.0897 [M+]) (Figure 3.14).
In the $^1$H NMR spectrum of 40c, the H-1' proton shows doublet at 5.09 ppm with $J = 7.8$ Hz, which exhibits (i) H,H-COSY correlation with the multiplet at 4.08-4.11 ppm, which is assignable to H-4 proton, (ii) C,H-COSY correlation with the signal at 50.8 ppm assignable to C-1' and (iii) HMBCs with C-5 at 26.0, C-4 at 44.1, C-3a at 157.5, C-1'' at 141.3, C-2'' at 128.0, C-4'' at 160.1 and C-5'' at 162.9 ppm (Figure 3.15). Further, H-4 proton shows C,H-COSY correlation with the signal at 44.1 ppm and HMBCs with C-5 at 26.0, C-6 at 20.4, C-3a at 157.5, C-1' at 50.8 and C-5'' at 162.9 ppm. The four cyclohexyl H-5 and H-6 protons appears as multiplet between 1.57-1.82 ppm, which give C,H-COSY correlation with the signal at 26.0 and 20.4 ppm, which is ascribable to C-5 and C-6 respectively. The axial like H-7 and equatorial like H-7 protons show two doublet of triplets at 2.78 ppm ($J = 18.0$ and 5.7 Hz) and 2.90 ppm ($J = 18.0$ and 6.0 Hz) respectively having (i) C,H-COSY correlation with the signal at 25.0 ppm due to C-7 and (ii) HMBC connections with C-6 at 20.4, C-5 at
26.0, C-7a at 158.6 and C-3a at 157.5 ppm. The mass spectrum for the compound 40e is obtained as m/e 498.9949 [M-1] (calcd. 500.0018 [M+]) (Figure 3.16 to 3.26).

**Figure 3.15.** Selected HMBCs and $^1$H and $^{13}$C chemical shifts in compound 40c

**Figure 3.16.** $^1$H NMR Spectrum of 40c (CDCl$_3$)
Figure 3.17. $^1$H NMR Spectrum of 40c (expanded)

Figure 3.18. $^{13}$C NMR Spectrum of 40c (CDCl$_3$)
Figure 3.19. DEPT Spectrum of 40c (CDCl₃)

Figure 3.20. H,H-COSY Spectrum of 40c
**Figure 3.21.** H,H-COSY Spectrum of 40c (expanded)

**Figure 3.22.** HMBC Spectrum of 40c
Figure 3.23. HMBC Spectrum of 40c (expanded)

Figure 3.24. HMBC Spectrum of 40c (expanded)
A mechanism for the formation of mono and bis-1,2,3-selenadiazoles is envisaged in Scheme 3.2. Initially, the condensation between 2-(3-oxo-1,3-diphenylpropyl)-1-cyclohexanone \(38\) and semicarbazide hydrochloride to afford the mono-semicarbazones \(41\). Then, the electrophilic addition of selenium (IV) oxide on \(41\) to give the selenic acid derivatives of \(42\), which is transformed into its enamine \(43\).
enamine 43, which on further cyclization, followed by the elimination of isocynanic acid furnishes the mono-1,2,3-selenadiazoles 39. Similarly, bis-1,2,3-selenadiazoles 40 is also formed from the bis-semicarbazones 46 via the same pathway.

\[
\begin{align*}
38 & \quad 39 \\
43 & \quad 44 \\
44 & \quad 46 \\
46 & \quad 39 \\
\end{align*}
\]

Scheme 3.2. Proposed mechanism for formation of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

3.4. Antibacterial activity of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

All the synthesized compounds are evaluated for their in vitro preliminary antibacterial activity against S. aureus (ATCC 11632) (Gram-positive bacteria), E. coli (ATCC 25922) (Gram-negative bacteria) and for their antifungal activity against C. albicans (ATCC 90028) by agar disc diffusion method using ciprofloxacin and clotrimazole as reference standards respectively.

The MICs and zone of inhibition were determined for 39a-k and 40a-k and the results are summarized along with that of ciprofloxacin and clotrimazole in Table 3.2. The results show that most of the designed compounds have moderate to good antibacterial and antifungal activities (6.25-50.0µg/ml). Compounds 39a, 39c, 39f, 39g, 39h, 39i, 39k, 40h and 40k show both moderate and good activity and have zone
of inhibition (18.0, 12.0, 18.0, 15.0, 20.0, 17.0, 20.0, 15.0 and 18.0 mm respectively) compared to that of the standard ciprofloxacin (6.25 μg/ml and 28 mm) against *E. coli*. Against *S. aureus*, compounds 39a, 39b, 39e, 39f, 39h, 39k and 40e show moderate activity (15, 15, 15, 16, 20, 20 and 16 mm zone of inhibition respectively) compared to the standard (6.25 μg/ml and 25 mm). In the antifungal evaluation, compounds 39b, 39g, 39h, 39g and 40h exhibit moderate activity (zone of inhibition 15, 17, 15, 15 and 15 mm respectively) compared to standard clotrimazole (6.25 μg/ml and 28 mm) against *C. albicans*.

**Table 3.2.** Antibacterial activity of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

<table>
<thead>
<tr>
<th>Entry</th>
<th>MIC(^a) (ZI)(^b)</th>
<th>E. coli (25922)</th>
<th>S. aureus (11632)</th>
<th>C. albicans (90028)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>39</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>a</td>
<td>12.5(18)</td>
<td>25.0(12)</td>
<td>12.5(15)</td>
<td>25.0(15)</td>
</tr>
<tr>
<td>b</td>
<td>25.0(10)</td>
<td>25.0(13)</td>
<td>12.5(15)</td>
<td>25.0(10)</td>
</tr>
<tr>
<td>c</td>
<td>12.5(12)</td>
<td>25.0(12)</td>
<td>25.0(15)</td>
<td>25.0(12)</td>
</tr>
<tr>
<td>d</td>
<td>25.0(10)</td>
<td>25.0(10)</td>
<td>25.0(15)</td>
<td>50.0(10)</td>
</tr>
<tr>
<td>e</td>
<td>25.0(&lt;10)</td>
<td>25.0(12)</td>
<td>12.5(15)</td>
<td>12.5(16)</td>
</tr>
<tr>
<td>f</td>
<td>12.5(18)</td>
<td>50.0(&lt;10)</td>
<td>12.5(16)</td>
<td>25.0(20)</td>
</tr>
<tr>
<td>g</td>
<td>12.5(15)</td>
<td>25.0(15)</td>
<td>25.0(18)</td>
<td>25.0(15)</td>
</tr>
<tr>
<td>h</td>
<td>6.25(20)</td>
<td>12.5(15)</td>
<td>6.25(20)</td>
<td>25.0(15)</td>
</tr>
<tr>
<td>i</td>
<td>12.5(17)</td>
<td>25.0(16)</td>
<td>25.0(18)</td>
<td>50.0(&lt;10)</td>
</tr>
<tr>
<td>j</td>
<td>25.0(15)</td>
<td>50.0(15)</td>
<td>25.0(18)</td>
<td>50.0(15)</td>
</tr>
<tr>
<td>k</td>
<td>12.5(20)</td>
<td>12.5(18)</td>
<td>12.5(20)</td>
<td>25.0(18)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>6.25(28)</td>
<td>6.25(25)</td>
<td>n.t.</td>
<td></td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>n.t.</td>
<td>n.t.</td>
<td>6.25(28)</td>
<td></td>
</tr>
</tbody>
</table>

Note: The MIC values were evaluated at concentration range 1.56-50μg/ml. \(^a\)Minimum inhibitory concentration in μg/mL. \(^b\)Zone of inhibition in mm for 100 μg/disc of each compound. \(^c\)n. t. not tested.

3.5. Antimycobacterial activity of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

All the synthesised compounds are also screened for their *in vitro* antimycobacterial activity against MTB in Middlebrook 7H11 agar medium supplemented with OADC by agar dilution method similar to that method recommended by the National
Committee for Clinical Laboratory Standards for the determination of MIC in duplicate. The MTB clinical isolate was resistant to isoniazid, rifampicin, ethambutol and ciprofloxacin. The minimum concentration of compound required to hinder 99\% of bacterial growth in the culture is referred as MIC and the corresponding values for the synthesized compounds at 7.4 pH are given in Table 3.3.

Table 3.3. Mycobacterium tuberculosis H37Rv (MTB) activities of mono and bis-1,2,3-selenadiazole derivatives 39 and 40.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compd.</th>
<th>Ar</th>
<th>Ar′</th>
<th>MTB (MIC) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a</td>
<td>C₆H₅</td>
<td>C₆H₅</td>
<td>15.8</td>
</tr>
<tr>
<td>2</td>
<td>b</td>
<td>C₆H₅</td>
<td>4-ClC₆H₄</td>
<td>7.2</td>
</tr>
<tr>
<td>3</td>
<td>c</td>
<td>C₆H₅</td>
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<td>29.3</td>
</tr>
<tr>
<td>4</td>
<td>d</td>
<td>C₆H₅</td>
<td>2-Naphthyl</td>
<td>28.0</td>
</tr>
<tr>
<td>5</td>
<td>e</td>
<td>4-McC₆H₄</td>
<td>C₆H₅</td>
<td>15.2</td>
</tr>
<tr>
<td>6</td>
<td>f</td>
<td>4-ClC₆H₄</td>
<td>C₆H₅</td>
<td>3.6</td>
</tr>
<tr>
<td>7</td>
<td>g</td>
<td>4-ClC₆H₄</td>
<td>4-McC₆H₄</td>
<td>3.5</td>
</tr>
<tr>
<td>8</td>
<td>h</td>
<td>4-ClC₆H₄</td>
<td>4-ClC₆H₄</td>
<td>3.3</td>
</tr>
<tr>
<td>9</td>
<td>i</td>
<td>C₆H₅</td>
<td>4-McC₆H₄</td>
<td>n. t.¹</td>
</tr>
<tr>
<td>10</td>
<td>j</td>
<td>4-MeOC₆H₄</td>
<td>C₆H₅</td>
<td>n. t.</td>
</tr>
<tr>
<td>11</td>
<td>k</td>
<td>4-ClC₆H₄</td>
<td>4-MeOC₆H₄</td>
<td>n. t.</td>
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<thead>
<tr>
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<th>39</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>7.6</td>
<td></td>
</tr>
</tbody>
</table>

¹ n. t. not tested.

In the first phase of screening against MTB, all the compounds show excellent in vitro activity with MIC ranging from 3.3 to 51.6 μM. Four compounds (39b, 39f, 39g and 39h) inhibits MTB with MIC less than 7.6 μM and are more potent than the first line anti-TB drug, ethambutol (MIC: 7.6 μM). When compared to ethambutol (MIC: 7.6 μM), one compound, 1-(4-chlorophenyl)-3-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone 39b (MIC: 7.2 μM) is found to be more potent against MTB. When compared to ciprofloxacin (MIC: 4.7 μM), three compounds 39f (MIC: 3.6 μM), 39g (MIC: 3.5 μM) and 39h (MIC: 3.3 μM) are found to be more potent against MTB. The other series of bis-1,2,3-selenadiazoles 40a-h, however, are less potent than rifampicin, isoniazid, ciprofloxacin and ethambutol. 1,3-Di(4-chlorophenyl)-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39h) is found to be the most active compound in vitro with MIC of 3.3 μM against MTB and is 1.42 and 2.30 times more potent than ciprofloxacin and ethambutal.
respectively. 3-(4-Chlorophenyl)-1-(4-methylphenyl)-3-(4,5,6,7-tetrahydro-1,2,3-benzoselena diazol-4-yl)-1-propanone (39g) and 3-(4-chlorophenyl)-1-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39f) are also found to be more active with MIC of 3.5 and 3.6 μM against MTB than ciprofloxacin and ethambutol respectively.

With respect to structure-MTB activity relationship, the data in Table 3.3 show that the mono-selenadiazoles (39a-h) possess more activity against MTB than the bis-selenadiazoles (40a-k), as revealed by the comparison of MIC data of compounds 39 and 40 (Figure 3.27).

![Figure 3.27. Comparison of MIC (μM) values of 39, 40 and standard drugs](image)

Among the aroyl and aryl groups in the series of compound 39, the order of activity is 4-ClC₆H₄ > 4-MeC₆H₄ > C₆H₅ > 2-Naphthyl > 4-MeOC₆H₄. In series of mono-1,2,3-selenadiazoles, the presence of chlorine atom in the aryl ring at para-position enhances the antimycobacterial activity as seen from the MIC values of 39f (MIC: 3.6 μM), 39g (MIC: 3.5 μM), 39h (MIC: 3.3 μM) (Table 3.3). 39h with p-chlorophenyl ring displays maximum activity against MTB.

### 3.6. Cytotoxicity

The cytotoxicity of the most active compound 39h was studied in vitro using NIH 3T3 mouse embryonic fibroblasts cell line (NIH 3T3) by MTT assay. MTT is a
yellow coloured water soluble tetrazolium salt. A mitochondrial enzyme in living
cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to
an insoluble purple formazan product that was read spectrophotometrically at 570 nm
on the basis of linear absorbance to the number of living cells in culture. The MTT
assay was validated using various concentrations of DMSO.

The dose-response graph given in (Figure 3.28) reveals that the percentage cell
viability decreases with increasing the concentration of 39h. However, the half
maximal inhibitory concentration (IC50) value determined by Graph Pad Prism
software is found to be 245 µM for 39h. This indicates that the most active of the
newly synthesized compounds, 39h, is not toxic to the normal fibroblasts (NIH 3T3).

![Figure 3.28. Log of concentration - percentage cell viability graph](image)

3.7. Conclusion
The synthesis of new sets of novel mono and bis-1,2,3-selenadiazole derivatives from
the semicarbazones of 2-(3-oxo-1,3-diarylpropyl)-1-cyclohexanones has been
described. Procedure for the selective synthesis of mono and bis-selenadiazoles has
been achieved by controlling the mole ratio of semicarbazide. All the synthesized
compounds were characterized by using NMR, mass and crystal analysis. These
1,2,3-selenadiazole derivatives displayed moderate to good in vitro antibacterial,
antifungal and antimycobacterial activities. The most active compound was tested for
cytotoxicity by using NIH 3T3 mouse embryonic fibroblast cell line. The most active
compound 39h was tested for cytotoxicity and it displayed less toxicity towards NIH
3T3 mouse embryonic fibroblast cell line. This lack of cytotoxic potential of
compounds 39h was of great significance for its possible use in the treatment of
microbial infections and tuberculosis.
3.8. Experimental section

3.8.1. General method

The melting points were measured in open capillary tubes and are uncorrected. The $^1$H, $^{13}$C and the 2D NMR spectra were recorded on a Bruker (Avance) 300 MHz NMR instrument using TMS as internal standard and CDCl$_3$ as solvent. Standard Bruker software was used throughout. Chemical shifts are given in parts per million (δ-scale) and the coupling constants are given in Hertz. Silica gel-G plates (Merck) were used for TLC analysis with a mixture of petroleum ether (60-80 °C) and ethylacetate as eluant. Elemental analyses were performed on a Perkin Elmer 2400 Series II Elemental CHNS analyzer. Functional groups were recorded on JASCO FT-IR-410 (4000-400cm$^{-1}$).

3.8.2. Synthesis of mono-1,2,3-selenadiazoles (39):

*General procedure:* A mixture of 2-(3-oxo-1,3-diarylpropyl)-1-cyclohexanone 38 (1 mmol), semicarbazide hydrochloride (1 mmol) and sodium acetate (1 mmol) in ethanol (10 mL) was refluxed for 3 h. After completion of the reaction as evident from TLC, the mixture was poured into ice water (50 mL) and the resultant solid was filtered off. Then, a mixture of this mono semicarbazone of 38 and selenium dioxide (2 mmol) in THF (10 ml) was refluxed on a waterbath for 30 min. After completion of the reaction as monitored by TLC, the reaction mixture was filtered to remove selenium powder, the filtrate was concentrated under vacuum, and the residue was subjected to column chromatography using petroleum ether/ethylacetate mixture (95:5; v/v) as eluant to afford the pure product 39. The analytical data for all the compounds are given below:

3.8.2.1. 1,3-Diphenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propnone (39a). Isolated as colorless solid; m.p. 120-121 °C; IR (KBr): 2928 (C-H), 1679 (C=O), 1593 (N=N), 1344 (C-N) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) δ$_H$: 1.65-1.80 (m, 3H, H-5 and one of H-6), 2.02-2.07 (m, 1H, H-6), 2.88 (dt, 1H, $J$ = 18.0, 6.3 Hz, H-7), 3.07 (dt, 1H, $J$ =18.0, 5.4 Hz, H-7), 3.61 (dd, 1H, $J$ = 18.6, 9.3 Hz, H-2'), 3.77-3.82 (m, 1H, H-4), 3.89-3.96 (m, 2H, H-2' & H-3'), 7.18-7.39 (m, 6H, Ar-H), 7.45-7.48 (m, 2H, Ar-H), 7.82 (d, 2H, $J$ = 7.5 Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ$_C$: 19.5, 24.7, 25.2, 39.9, 43.4, 44.6, 126.5, 127.9, 128.2, 128.3, 128.4, 132.7, 137.1, 143.1, 157.7,
159.8, 198.7. Anal. Calcd. for C_{21}H_{20}N_{2}OSe: C, 63.80; H, 5.10; N, 7.09%. Found: C, 63.76; H, 5.07; N, 7.13%.

3.8.2.2. 1-(4-Chlorophenyl)-3-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39b). Isolated as colorless solid; m.p. 122–123 °C; IR (KBr): 2927 (C-H), 1673 (C=O), 1589 (N=N), 1354 (C-N) cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ_H: 1.61-1.84 (m, 3H, H-5 and one of H-6), 1.98-2.11 (m, 1H, H-6), 2.89 (dt, 1H, J = 18.3, 6.3 Hz, H-7), 3.12 (dt, 1H, J = 18.3, 5.7 Hz, H-7), 3.52 (dd, 1H, J = 16.5, 5.6 Hz, H-2'), 3.74-3.80 (m, 1H, H-2' & H-3'), 7.17-7.32 (m, 5H, Ar-H), 7.34 (d, 2H, J = 8.7 Hz, Ar-H), 7.75 (d, 2H, J = 8.7 Hz, Ar-H); ^13C NMR (75 MHz, CDCl3) δ_C: 19.4, 24.8, 25.2, 39.9, 43.5, 44.6, 126.6, 128.2, 128.5, 128.6, 129.3, 135.4, 139.1, 143.1, 157.8, 159.8, 197.6. Anal. Calcd. for C_{21}H_{19}ClN_{2}OSe: C, 58.68; H, 4.46; N, 6.52%. Found: C, 58.63; H, 4.42; N, 6.56%.

3.8.2.3. 1-(4-Methoxyphenyl)-3-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39c). Isolated as colorless solid; m.p. 112-113 °C; IR (KBr): 2938 (C-H), 1668 (C=O), 1598 (N=N), 1353 (C-N) cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ_H: 1.65-1.80 (m, 3H, H-5 and one of H-6), 2.01-2.10 (m, 1H, H-6), 2.89 (dt, 1H, J = 18.0, 6.6 Hz, H-7), 3.08 (dt, 1H, J = 18.0, 5.1 Hz, H-7), 3.55 (dd, 1H, J = 17.1, J = 7.8 Hz, H-2'), 3.75-3.89 (m, 5H, OCH_3, H-2' & H-4), 3.93-3.97 (m, 1H, H-3'), 6.85 (d, 2H, J = 8.7 Hz, Ar-H), 7.18-7.34 (m, 5H, Ar-H), 7.82 (d, 2H, J = 8.7 Hz, Ar-H); ^13C NMR (75 MHz, CDCl3) δ_C: 19.5, 24.8, 25.2, 39.9, 43.5, 44.6, 126.6, 128.2, 128.6, 129.3, 135.4, 139.1, 143.1, 157.8, 159.9, 163.2, 197.2. Anal. Calcd. for C_{22}H_{22}N_{2}O_{2}Se: C, 62.12; H, 5.21; N, 6.59%. Found: C, 62.07; H, 5.17; N, 6.63%.

3.8.2.4. 1-(2-Naphthyl)-3-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39d). Isolated as colorless solid; m.p. 131-132 °C; IR (KBr): 2937 (C-H), 1675 (C=O), 1596 (N=N), 1349 (C-N) cm^{-1}; ^1H NMR (300 MHz, CDCl3) δ_H: 1.64-1.82 (m, 3H, H-5 and one of H-6), 2.04-2.10 (m, 1H, H-6), 2.91(dt, 1H, J = 18.0, 6.3 Hz, H-7), 3.11 (dt, 1H, J = 18.3, 5.4 Hz, H-7), 3.74 (dd, 1H, J = 16.5, 7.2 Hz, H-2'), 3.80-3.85 (m, 1H, H-4), 3.94-4.09 (m, 2H, H-2' & H-3'), 7.17-7.37 (m, 5H, Ar-H), 7.49-7.58 (m, 2H, Ar-H), 7.78-7.88 (m, 3H, Ar-H), 7.92 (d, 1H, J = 7.5 Hz, Ar-H), 8.38 (s, 1H, Ar-H); ^13C NMR (75 MHz, CDCl3) δ_C: 19.5, 24.9, 25.3, 40.0,
43.5, 44.9, 123.8, 126.6 (2C), 127.6, 128.2, 128.3 (2C), 128.5 (2C), 129.6, 132.4, 134.4, 135.4, 143.2, 157.8, 159.9, 198.7. Anal. Calcd. for \( \text{C}_{25}\text{H}_{22}\text{N}_{2}\text{OSe} \): C, 67.41; H, 4.98; N, 6.29%. Found: C, 67.38; H, 4.94; N, 6.34%.

3.8.2.5. 3-(4-Methylphenyl)-1-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39e). Isolated as colorless solid; m.p. 125-126 °C; IR (KBr): 2940 (C-H), 1679 (C=O), 1585 (N=N), 1351 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\) \(\delta_h\)): 1.65-1.81 (m, 3H, H-5 and one of H-6), 2.01-2.08 (m, 1H, H-6), 2.30 (s, 3H, CH\(_3\)), 2.89 (dt, 1H, \(J = 18.0, 6.9\) Hz, H-7), 3.08 (dt, 1H, \(J = 18.0, 5.4\) Hz, H-7), 3.58 (dd, 1H, \(J = 18.6, 9.3\) Hz, H-2'), 3.74-3.80 (m, 1H, H-4), 3.86-3.94 (m, 2H, H-2' & H-3'), 7.10 (d, 2H, \(J = 7.8\) Hz, Ar-H), 7.21 (d, 2H, \(J = 7.8\) Hz, Ar-H), 7.35-7.40 (m, 2H, Ar-H), 7.46-7.51 (m, 1H, Ar-H), 7.84 (d, 2H, \(J = 8.4\) Hz, Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\) \(\delta_c\)): 19.6, 21.0, 24.7, 25.2, 40.0, 43.5, 44.2, 127.9, 128.1, 128.3, 129.1, 132.7, 136.0, 137.1, 140.0, 157.7, 159.9, 198.8. Calcd. for \(m/z\): 410.0897 [M\(^+\)]; Found: 409.0824 [M-1]; Anal. Calcd. for \(\text{C}_{22}\text{H}_{22}\text{N}_{2}\text{OSe} \): C, 64.54; H, 5.42; N, 6.84%. Found: C, 64.50; H, 5.39; N, 6.89%.

3.8.2.6. 3-(4-Chlorophenyl)-1-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39f). Isolated as colorless solid; m.p. 115-116 °C; IR (KBr): 2925 (C-H), 1675 (C=O), 1589 (N=N), 1344 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\) \(\delta_h\)): 1.61-1.81 (m, 3H, H-5 and one of H-6), 1.95-2.09 (m, 1H, H-6), 2.91 (dt, 1H, \(J = 18.0, 6.9\) Hz, H-7), 3.06 (dt, 1H, \(J = 18.0, 5.1\) Hz, H-7), 3.58 (dd, 1H, \(J = 17.1, 8.1\) Hz, H-2'), 3.71-3.77 (m, 1H, H-4), 3.89 (dd, 1H, \(J = 17.1, 5.1\) Hz, H-2'), 3.93-3.98 (m, 1H, H-3'), 7.25 (s, 4H, Ar-H), 7.40 (d, 2H, \(J = 7.2\) Hz, Ar-H), 7.45-7.52 (m, 1H, Ar-H), 7.83 (d, 2H, \(J = 7.2\) Hz, Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\) \(\delta_c\)): 19.7, 24.7, 25.2, 39.9, 43.2, 44.0, 127.9, 128.1, 128.4, 128.6, 129.7, 132.9, 136.9, 141.5, 157.9, 159.5, 198.4. Anal. Calcd. for \(\text{C}_{22}\text{H}_{19}\text{ClN}_{2}\text{OSe} \): C, 58.68; H, 4.46; N, 6.52%. Found: C, 58.64; H, 4.41; N, 6.56%.

3.8.2.7. 3-(4-Chlorophenyl)-1-(4-methylphenyl)-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39g). Isolated as colorless solid; m.p. 116-117 °C; IR (KBr): 2925 (C-H), 1683 (C=O), 1594 (N=N), 1346 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\) \(\delta_h\)): 1.59-1.80 (m, 3H, H-5 and one of H-6), 1.94-2.04 (m, 1H, H-6), 2.36 (s, 3H, CH\(_3\)), 2.91 (dt, 1H, \(J = 18.0, 6.6\) Hz, H-7), 3.05 (dt, 1H, \(J = 18.0, 5.1\) Hz, H-2' & H-3'), 7.25 (s, 4H, Ar-H), 7.40 (d, 2H, \(J = 7.2\) Hz, Ar-H), 7.45-7.52 (m, 1H, Ar-H), 7.83 (d, 2H, \(J = 7.2\) Hz, Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\) \(\delta_c\)): 19.7, 24.7, 25.2, 39.9, 43.2, 44.0, 127.9, 128.1, 128.4, 128.6, 129.7, 132.9, 136.9, 141.5, 157.9, 159.5, 198.4. Anal. Calcd. for \(\text{C}_{22}\text{H}_{22}\text{ClN}_{2}\text{OSe} \): C, 58.68; H, 4.46; N, 6.52%. Found: C, 58.64; H, 4.41; N, 6.56%.
Hz, H-7), 3.56 (dd, 1H, J = 17.1, 8.4 Hz, H-2'), 3.70-3.76 (m, 1H, H-4), 3.83 (dd, 1H, J = 17.1, 5.4 Hz, H-2'), 3.96 (td, 1H, J = 8.4, 5.4 Hz, H-3'), 7.19 (d, 2H, J = 8.1 Hz, H-3", Ar-H), 7.24 (s, 4H, Ar-H), 7.74 (d, 2H, J = 8.1 Hz, H-2", Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ$_{C}$: 19.7, 21.5, 24.6, 25.2, 39.9, 42.9, 44.0, 128.0, 128.5, 129.1, 129.7, 132.1, 134.1, 141.5, 143.7, 157.8, 159.5, 198.0. Anal. Calcd. for C$_{22}$H$_{21}$ClN$_{2}$OSe: C, 59.54; H, 4.77; N, 6.31%. Found: C, 59.51; H, 4.73; N, 6.35%.

3.8.2.8. 1,3-Di-(4-chlorophenyl)-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39h). Isolated as colorless solid; m.p. 120-121 °C; IR (KBr): 2944 (C-H), 1675 (C=O), 1592 (N=N), 1354 (C-N) cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) δ$_{H}$: 1.61-1.79 (m, 3H, H-5 and one of H-6), 2.01-2.06 (m, 1H, H-6), 2.63-2.73 (m, 1H, H-7), 2.99-3.05 (m, 1H, H-7), 3.62 (dd, 1H, J = 17.4, 8.4 Hz, H-2'), 3.66-3.72 (m, 1H, H-4), 3.85 (dd, 1H, J = 17.4, 6.0 Hz, H-2'), 4.17-4.23 (m, 1H, H-3'), 6.98 (d, 2H, J = 8.7 Hz, Ar-H), 7.15 (d, 2H, J = 8.4 Hz, Ar-H), 7.41 (d, 2H, J = 8.7 Hz, Ar-H) 7.91 (d, 2H, J = 8.4 Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ$_{C}$: 21.9, 25.3, 26.3, 41.3, 41.8, 45.3, 128.3, 128.8, 129.6, 130.2, 132.4, 135.2, 139.5, 139.9, 158.5, 158.6, 197.8. Anal. Calcd. for C$_{21}$H$_{18}$Cl$_2$N$_{2}$OSe: C, 54.33; H, 3.91; N, 6.03%. Found: C, 54.30; H, 3.87; N, 6.07%.

3.8.2.9. 1-(4-Methylphenyl)-3-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39i). Isolated as colorless solid; Yield: m.p. 125-126 °C; IR (KBr): 2926 (C-H), 1673 (C=O), 1588 (N=N), 1354 (C-N) cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) δ$_{H}$: 1.64-1.81 (m, 3H, H-5 and one of H-6), 2.03-2.08 (m, 1H, H-6), 2.36 (s, 3H, CH$_3$), 2.89 (dt, 1H, J = 18.3, 6.3 Hz, H-7), 3.11 (dt, 1H, J = 18.3, 5.4 Hz, H-7), 3.56 (dd, 1H, J = 16.5, 7.2 Hz, H-2'), 3.77-3.80 (m, 1H, H-4), 3.83-3.99 (m, 2H, H-2' & H-3'), 7.18-7.39 (m, 7H, Ar-H), 7.73 (d, 2H, J = 8.7 Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ$_{C}$: 21.9, 25.3, 26.3, 41.3, 41.8, 45.3, 128.3, 128.8, 129.6, 130.2, 132.4, 135.2, 139.5, 139.9, 158.5, 158.6, 197.8. Anal. Calcd. for C$_{22}$H$_{22}$N$_{2}$OSe: C, 64.54; H, 5.42; N, 6.84%. Found: C, 64.50; H, 5.39; N, 6.89%.

3.8.2.10. 3-(4-Methoxyphenyl)-1-phenyl-3-(4,5,6,7-tetrahydro-1,2,3-benzoselenadiazol-4-yl)-1-propanone (39j). Isolated as colorless solid; m.p. 118-119 °C; IR (KBr): 2924 (C-H), 1669 (C=O), 1588 (N=N), 1344 (C-N) cm$^{-1}$; $^{1}$H NMR (300 MHz, CDCl$_3$) δ$_{H}$: 1.67-1.76 (m, 3H, H-5 and one of H-6), 1.99-2.03 (m, 1H, H-
6), 2.89 (dt, 1H, \(J = 18.0, 6.6\) Hz, H-7), 3.08 (dt, 1H, \(J = 18.0, 5.1\) Hz, H-7), 3.56 (dd, 1H, \(J = 17.1, 7.8\) Hz, H-2'), 3.73 (s, 3H, OCH\(_3\)), 3.74-3.87 (m, 2H, H-2' & H-4), 3.96-3.98 (m, 1H, H-4), 6.81 (d, 2H, \(J = 8.7\) Hz, Ar-H), 7.20-7.52 (m, 5H, Ar-H), 7.82 (d, 2H, \(J = 8.7\) Hz, Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\)C: 19.7, 24.6, 25.2, 40.1, 43.5, 43.9, 55.0, 113.7, 127.8, 128.3, 128.4, 132.6, 134.9, 137.2, 157.5, 158.1, 159.8, 198.8. Anal. Calcd. for C\(_{22}\)H\(_{22}\)N\(_2\)O\(_2\)Se: C, 62.12; H, 5.21; N, 6.59%. Found: C, 62.08; H, 5.18; N, 6.63%.

3.8.2.11. 3-(4-Chlorophenyl)-1-(4-methoxyphenyl)-3-(4,5,6,7-tetrahydro-1,2,3-benzo selenadiazol-4-yl)-1-propanone (39k). Isolated as colorless solid; m.p. 124-125 °C; IR (KBr): 2926 (C-H), 1688 (C=O), 1596 (N=N), 1349 (C=N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\)H: 1.64-1.77 (m, 3H, H-5 and one of H-6), 2.03-2.08 (m, 1H, H-6), 2.91 (dt, 1H, \(J = 18.0, 6.6\) Hz, H-7), 3.05 (dt, 1H, \(J = 18.0, 5.1\) Hz, H-7), 3.56 (dd, 1H, \(J = 17.1, 8.4\) Hz, H-2'), 3.70-3.76 (m, 1H, H-4), 3.83-3.87 (m, 4H, H-2', OCH\(_3\)), 3.96 (m, 1H, H-3'), 6.85 (d, 2H, \(J = 8.1\) Hz, Ar-H), 7.30-7.35 (m, 4H, Ar-H), 7.82 (d, 2H, \(J = 8.1\) Hz, Ar-H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\)C: 19.8, 24.8, 25.2, 40.0, 42.7, 44.2, 55.3, 113.5, 128.6, 129.1, 129.7, 130.3, 130.7, 141.6, 157.8, 159.5, 163.3, 196.9. Anal. Calcd. for C\(_{22}\)H\(_{21}\)ClN\(_2\)O\(_2\)Se: C, 57.46; H, 4.60; N, 6.09%. Found: C, 57.42; H, 4.57; N, 6.14%.

3.8.3. Synthesis of bis-1,2,3-selenadiazoles (40):

**General procedure:** A mixture of 2-(3-oxo-1,3-diarylpropyl)-1-cyclohexanone 38 (1 mmol), semicarbazide hydrochloride (2.5 mmol) and sodium acetate (2.5 mmol) in ethanol (10 mL) was heated under reflux on a water bath for 3 h. After completion of the reaction as evident from TLC, the mixture was poured into ice-water (50 mL) and the resulting mixture of semicarbazones was filtered off. Then, this mixture of semicarbazones of 38 and selenium dioxide (3 mmol) in THF (10 ml) was refluxed on a water bath for 30 min. After completion of the reaction as monitored by TLC, the reaction mixture was filtered to remove selenium powder, the filtrate was concentrated under vacuum, and the residue was subjected to column chromatography using petroleum ether/ethylacetate mixture (95:5; v/v) as eluant to afford the pure products 39 and 40. Analytical data for 40 are given below:
3.8.3.1. 4-[Phenyl(4-phenyl-1,2,3-selenadiazol-5-yl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40a). Isolated as pale green solid; Yield: m.p. 124-125 °C; IR (KBr): 2935 (C-H), 1580 (N-N), 1325 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 1.56-1.77 (m, 4H, H-5, H-6), 2.73 (dt, 1H, \(J = 18.0, 5.4\) Hz, H-7), 2.86 (dt, 1H, \(J = 18.0, 6.3\) Hz, H-7), 4.12 (dt, 1H, \(J = 8.1, 5.1\) Hz, H-4), 5.05 (d, 1H, \(J = 8.1\) Hz, H-1'), 7.21-7.29 (m, 5H, Ar-H), 7.32-7.38 (m, 5H, Ar-H); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 20.1, 24.9, 25.9, 43.8, 50.5, 127.4, 127.8, 128.4, 128.5, 129.1, 129.5, 132.0, 141.3, 157.3, 158.6, 160.0, 163.9. Anal. Calcd. for C\(_{21}\)H\(_{18}\)N\(_4\)Se\(_2\): C, 52.08; H, 3.75; N, 11.57%. Found: C, 52.05; H, 3.71; N, 11.61%.

3.8.3.2. 4-[(4-(4-Chlorophenyl)-1,2,3-selenadiazol-5-yl)(phenyl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40b). Isolated as pale green solid; m.p. 142-143 °C; IR (KBr): 2948 (C-H), 1589 (N-N), 1340 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 1.59-1.82 (m, 4H, H-5, H-6), 2.79 (dt, 1H, \(J = 18.3, 5.4\) Hz, H-7), 2.92 (dt, 1H, \(J = 18.3, 6.0\) Hz, H-7), 4.06-4.12 (m, 1H, H-4), 5.01 (d, 1H, \(J = 8.1\) Hz, H-1'), 7.18-7.22 (m, 4H, Ar-H), 7.30-7.40 (m, 5H, Ar-H); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 20.1, 24.9, 25.9, 43.8, 50.9, 127.6, 127.9, 128.7, 129.2, 130.7, 131.0, 134.6, 140.9, 157.4, 158.7, 158.9, 164.4. Anal. Calcd. for C\(_{21}\)H\(_{17}\)ClN\(_4\)Se\(_2\): C, 48.62; H, 3.30; N, 10.80%. Found: C, 48.59; H, 3.26; N, 10.84%.

3.8.3.3. 4-[(4-(4-Methoxyphenyl)-1,2,3-selenadiazol-5-yl)(phenyl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40c). Isolated as pale green solid; m.p. 123-124 °C; IR (KBr): 2930 (C-H), 1531 (N=N), 1349 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 1.57-1.82 (m, 4H, H-5, H-6), 2.78 (dt, 1H, \(J = 18.0, 5.7\) Hz, H-7), 2.91 (dt, 1H, \(J = 18.0, 6.0\) Hz, Ar-H, H-7), 3.86 (s, 3H, OCH\(_3\)), 4.08-4.11 (m, 1H, H-4), 5.09 (d, 1H, \(J = 7.8\) Hz, H-1'), 6.90 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.18 (d, 2H, \(J = 8.4\) Hz, Ar-H), 7.21-7.41 (m, 5H, Ar-H); \(^1\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\): 20.4, 25.0, 26.0, 44.3, 50.9, 127.6, 127.9, 128.7, 129.2, 130.7, 131.0, 134.6, 140.9, 157.4, 158.9, 158.9, 164.4. Anal. Calcd. for C\(_{22}\)H\(_{20}\)N\(_4\)OSe\(_2\): C, 51.37; H, 3.92; N, 10.89%. Found: C, 51.33; H, 3.88; N, 10.94%.

3.8.3.4. 4-[(4-(2-Naphthyl)-1,2,3-selenadiazol-5-yl)(phenyl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40d). Isolated as pale green solid; m.p. 134-135 °C; IR (KBr): 2943 (C-H), 1589 (N=N), 1326 (C-N) cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\):
δH: 1.63-1.79 (m, 4H, H-5, H-6), 2.67 (dt, 1H, J = 18.0, 6.0 Hz, H-7), 2.85 (dt, 1H, J = 18.0, 6.0 Hz, H-7), 4.11-4.17 (m, 1H, H-4), 5.14 (d, 1H, J = 8.1 Hz, H-1'), 7.25-7.55 (m, 8H, Ar-H), 7.61 (s, 1H, Ar-H), 7.72-7.75 (m, 1H, Ar-H), 7.86-7.89 (m, 2H, Ar-H); 13C NMR (75 MHz, CDCl3) δC: 20.2, 25.0, 26.0, 44.1, 51.0, 126.4, 126.6, 127.2, 127.5, 127.7, 128.1(2C), 128.2 (2C), 129.1, 129.2, 129.6, 133.0, 141.4, 157.4, 158.7, 160.1, 164.1. Anal. Calcd. for C25H20N4Se2: C, 56.19; H, 3.77; N, 10.48%. Found: C, 56.15; H, 3.73; N, 10.51%.

3.9.3.5. 4-[4-Methylphenyl](4-phenyl-1,2,3-selenadiazol-5-yl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40e). Isolated as pale green solid; m.p. 136-137 °C; IR (KBr): 2925 (C-H), 1580 (N=N), 1322 (C-N) cm −1; 1H NMR (300 MHz, CDCl3) δH: 1.59-1.77 (m, 4H, H-5, H-6), 2.34 (s, 3H, CH3), 2.75 (dt, 1H, J = 18.0, 5.1 Hz, H-7), 2.85 (dt, 1H, J = 18.0, 6.6 Hz, H-7), 4.06-4.10 (m, 1H, H-4), 5.03 (d, 1H, J = 8.4 Hz, H-1'), 7.12-7.15 (m, 4H, Ar-H), 7.22-7.26 (m, 2H, Ar-H), 7.36-7.38 (m, 3H, Ar-H); 13C NMR (75 MHz, CDCl3) δC: 20.1, 21.0, 24.9, 25.9, 43.9, 50.2, 127.7, 128.4 (2C), 129.6, 129.8, 132.1, 137.2, 138.4, 157.4, 158.5, 160.0, 164.3. Calcd. for (m/z): 500.0018 [M+]; Found: 498.9949 [M-1]; Anal. Calcd. for C22H20N4Se2: C, 53.02; H, 4.05; N, 11.24%. Found: C, 52.97; H, 4.01; N, 11.28%.

3.8.3.6. 4-[4-Chlorophenyl](4-phenyl-1,2,3-selenadiazol-5-yl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40f). Isolated as pale green solid; m.p. 132-133 °C; IR (KBr): 2950 (C-H), 1586 (N=N), 1325 (C-N) cm −1; 1H NMR (300 MHz, CDCl3) δH: 1.66-1.80 (m, 4H, H-5, H-6), 2.71 (dt, 1H, J = 18.3, 6.0 Hz, H-7), 2.90 (dt, 1H, J = 18.3, 5.7 Hz, H-7), 4.02-4.15 (m, 1H, H-4), 5.11 (d, 1H, J = 7.2 Hz, H-1'), 7.14 (d, 2H, J = 8.4 Hz, Ar-H), 7.24-7.26 (m, 2H, Ar-H), 7.29 (d, 2H, J = 8.4 Hz, Ar-H), 7.36-7.40 (m, 3H, Ar-H); 13C NMR (75 MHz, CDCl3) δC: 20.5, 24.9, 26.1, 43.9, 50.0, 128.5, 128.6, 129.1, 129.3, 129.5, 131.9, 133.1, 139.6, 157.0, 159.1, 160.4, 162.5. Anal. Calcd. for C21H17ClN4Se2: C, 48.62; H, 3.30; N, 10.80%. Found: C, 48.59; H, 3.26; N, 10.85%.

3.8.3.7. 4-(4-Chlorophenyl)[4-(4-methylphenyl)-1,2,3-selenadiazol-5-yl]methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40g). Isolated as pale green solid; m.p. 174-175 °C; IR (KBr): 2927 (C-H), 1583 (N=N), 1336 (C-N) cm −1; 1H NMR (300 MHz, CDCl3) δH: 1.71-1.84 (m, 4H, H-5, H-6), 2.39 (s, 3H, CH3), 2.74 (dt, 1H, J
= 18.3, 5.4 Hz, H-7), 2.95 (dt, 1H, J = 18.3, 5.4 Hz, H-7), 4.06 (m, 1H, H-4), 5.16 (d, 1H, J = 6.9 Hz, H-1'), 7.12-7.16 (m, 4H, Ar-H), 7.20 (d, 2H, J = 8.7 Hz, Ar-H), 7.29, (d, 2H, J = 8.7 Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 20.7, 21.3, 25.0, 26.2, 44.1, 50.2, 129.0, 129.1, 129.2, 129.4, 129.5, 133.1, 138.6, 140.0, 157.1, 159.1, 160.1, 161.7. Anal. Calcd. for C$_{22}$H$_{19}$ClN$_4$Se$_2$: C, 49.60; H, 3.59; N, 10.52%. Found: C, 49.56; H, 3.55; N, 10.56%.

3.8.3.8. 4-(4-Chlorophenyl)[4-(4-chlorophenyl)-1,2,3-selenadiazol-5-yl]methyl-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40h). Isolated as pale green solid; m.p. 155-156 °C; IR (KBr): 2938 (C-H), 1587 (N=N), 1328 (C-N) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.72-1.88 (m, 4H, H-5, H-6), 2.78 (dt, 1H, J = 18.0, 5.7 Hz, H-7), 2.98 (dt, 1H, J = 18.0, 5.7 Hz, H-7), 4.02-4.16 (m, 1H, H-4), 5.09 (d, 1H, J = 6.9 Hz, H-1'), 7.11 (d, 2H, J = 8.4 Hz, Ar-H), 7.22 (d, 2H, J = 8.4 Hz, Ar-H), 7.31 (d, 2H, J = 8.4 Hz, Ar-H), 7.38 (d, 2H, J = 8.4 Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 20.8, 25.1, 26.2, 44.4, 50.4, 128.9, 129.3, 129.4, 130.5, 130.9, 133.4, 134.9, 139.3, 157.1, 159.1, 159.3, 162.9. Anal. Calcd. for C$_{21}$H$_{16}$Cl$_2$N$_4$Se$_2$: C, 45.59; H, 2.92; N, 10.13%. Found: C, 45.55; H, 2.87; N, 10.17%.

3.8.3.9. 4-[4-(4-Methylphenyl)-1,2,3-selenadiazol-5-yl](phenyl)methyl-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40i). Isolated as pale green solid; m.p. 148-149 °C; IR (KBr): 2949 (C-H), 1588 (N=N), 1340 (C-N) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.62-1.81 (m, 4H, H-5, H-6), 2.38 (s, 3H, CH$_3$), 2.76 (dt, 1H, J = 18.0, 5.4 Hz, H-7), 2.89 (dt, 1H, J = 18.0, 6.3 Hz, H-7), 4.07-4.13 (m, 1H, H-4), 5.09 (d, 1H, J = 8.1 Hz, H-1'), 7.11-7.19 (m, 4H, Ar-H), 7.22-7.37 (m, 5H, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 20.8, 25.1, 26.2, 44.4, 50.4, 129.0, 129.1, 129.2, 129.5, 138.4, 141.4, 157.4, 158.6, 160.3, 163.2. Anal. Calcd. for C$_{22}$H$_{20}$Cl$_2$N$_4$Se$_2$: C, 53.02; H, 4.05; N, 11.24%. Found: C, 52.97; H, 4.01; N, 11.28%.

3.8.3.10. 4-[(4-Methoxyphenyl)(4-phenyl-1,2,3-selenadiazol-5-yl)methyl]-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40j). Isolated as pale green solid; m.p. 136–137 °C; IR (KBr): 2937 (C-H), 1587 (N=N), 1326 (C-N) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 1.59-1.79 (m, 4H, H-5, H-6), 2.75 (dt, 1H, J = 18.0, 5.7 Hz, H-7), 2.88 (dt, 1H, J = 18.0, 6.0 Hz, H-7), 3.80 (s, 3H, OCH$_3$), 4.04-4.09 (m, 1H, H-4), 5.05 (d, 1H, J = 8.1 Hz, H-1'), 6.87 (d, 2H, J = 8.4 Hz, Ar-H), 7.14 (d, 2H, J = 8.4 Hz, Ar-H), 7.24-
7.26 (m, 2H, Ar-H), 7.37-7.39 (m, 3H, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$C: 20.3, 24.9, 25.9, 44.0, 49.8, 55.2, 114.4, 128.4, 129.0, 129.6 (2C), 132.2, 133.4, 157.5, 158.5, 158.7, 159.9, 164.3. Anal. Calcd. for C$_{22}$H$_{20}$N$_4$OSe$_2$: C, 51.37; H, 3.92; N, 10.89%. Found: C, 51.33; H, 3.88; N, 10.93%.

3.8.3.11. 4-(4-Chlorophenyl)[4-(4-methoxyphenyl)-1,2,3-selenadiazol-5-yl]methyl-4,5,6,7-tetrahydro-1,2,3-benzoselenadiazole (40k). Isolated as pale green solid; m.p. 132-133 °C; IR (KBr): 2928 (C-H), 1585 (N=N), 1336 (C-N) cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$H: 1.70-1.82 (m, 4H, H-5, H-6), 2.73 (dt, 1H, $J = 18.0, 6.3$ Hz, H-7), 2.93 (dt, 1H, $J = 18.0, 5.7$ Hz, H-7), 3.81 (s, 3H, OCH$_3$), 4.00-4.07 (m, 1H, H-4), 5.13 (d, 1H, $J = 7.2$ Hz, H-1'), 6.89 (d, 2H, $J = 8.7$ Hz, Ar-H), 7.12 (d, 2H, $J = 8.4$ Hz, Ar-H), 7.18, (d, 2H, $J = 8.7$ Hz, Ar-H), 7.27, (d, 2H, $J = 8.4$ Hz, Ar-H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$C: 20.8, 25.1, 26.2, 44.2, 50.3, 55.3, 114.0, 124.4, 129.1, 129.5, 130.9, 133.2, 139.6, 157.2, 159.2, 159.8, 160.5, 161.2. Anal. Calcd. for C$_{22}$H$_{19}$ClN$_4$OSe$_2$: C, 48.15; H, 3.49; N, 10.21%. Found: C, 48.11; H, 3.46; N, 10.25%.

3.8.4. Antibacterial and antifungal activity

The compounds 39 and 40 were diluted in dimethylsulfoxide (DMSO) with required concentrations (100μg/disc) for bioassay. Antimicrobial activity was evaluated by screening the compounds by standard agar disc diffusion method against a panel of human pathogenic microorganisms: one Gram positive (S. aureus ATCC 11632) and one Gram negative (E. coli ATCC 25922) bacteria were used for the antibacterial assay, while for the antifungal assay, C. albicans (ATCC 90028) was used. Microorganisms were maintained at 37 °C on Mueller Hinton (MH) agar slants. MH agar and sabouraud’s broth were used to evaluate antibacterial and antifungal activity respectively. To make a judgment of antibacterial and antifungal potency of the synthesized compounds, commercial antibiotics such as ciprofloxacin (10μg/disc) and clotrimazole (10μg/disc) in DMSO served as reference standards to compare inhibition of growth. The plates containing bacterial organism were incubated at 37 °C for 24 h and the plates containing fungal organism were incubated at 28 °C for 48h. The zone of inhibition was calculated by measuring the diameter of zone of inhibition for bacterial and fungal growth around the disc. Averages of three independent determinations were recorded. The Minimum Inhibitory Concentrations (MIC) of the samples were determined by agar dilution method. MH broth was
moulled and poured in sterile tubes according to National Committee for Clinical Laboratory Standards (NCCLS, M7-A5 January 2000). Overnight culture were grown at 37 °C by Kirby- Bauer procedure and diluted to Muller Hinton Broth. 0.01ml of culture was added to all the test tubes containing serial double dilution of drugs. All the tubes were incubated at 37 °C for 18-24 h. After incubation, the OD values were observed by spectrophotometric method. The Minimum Inhibitory Concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth.

3.8.5. Anti-tubercular activity

Ten fold serial dilutions of each test compounds 39a-h and 40a-k was incorporated into Middle brook 7H11 agar medium with OADC Growth Supplement. Inoculum of M. tuberculosis H37Rv were prepared from fresh Middle brook 7H11 agar slants with OADC Growth Supplement adjusted to 1mg/mL (wet weight) in Tween 80 (0.05%) saline diluted to 10⁻² to give a concentration of approximately 10⁷ cfu/mL. A 5 μL amount of bacterial suspension was spotted into 7H11 agar tubes containing 10-fold serial dilutions of drugs per mL. The tubes were incubated at 37 °C, and final readings were recorded after 28 days. The minimum concentration of compound required to give complete inhibition of bacterial growth in each tube was determined as the MIC value for corresponding compound.

3.8.6. Cytotoxicity

The NIH 3T3 mouse embryonic fibroblasts line (NIH 3T3) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Dulbeccos Modified Eagles Medium containing 10% fetal bovine serum (FBS). All the cells were maintained at 37 °C, 5% CO₂, 95% air and 100% relative humidity. Maintenance cultures were passage weekly, and the culture medium was changed twice a week.

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of 1x10⁵ cells/ml. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37 °C, 5% CO₂, 95% air and 100% relative humidity. After 24 h, the cells were treated
with serial concentrations of the compound 39h. That was initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce various concentrations. One hundred microlitres per well of each concentration were added to plates to obtain final concentrations of 1000, 500, 250, 125 and 63 µM. The final volume in each well was 200µl and the plates were incubated at 37 °C, 5% CO₂, 95% air and 100% relative humidity for 48h. The medium without samples was served as control. Triplicate was maintained for all concentrations. After 48 h, the cells in each well were quantified by MTT assay. Briefly, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37 °C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The percentage cell viability was then calculated with respect to positive control as follows:

\[
\% \text{ cell viability} = \frac{[A] \text{ Test}}{[A] \text{ Control}} \times 100
\]


25. Crystallographic data (excluding structure factors) for 39b has been deposited with the Cambridge Crystallographic Data Centre as CCDC 804861.